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INTERACTIONS OF INSULIN AND ADRENALINE WITH GLYCEROL PHOSPHATE ACYLATION PROCESSES IN FAT-CELLS FROM RAT

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1. Introduction

Recent studies [1–8] have indicated that insulin and adrenaline may cause acute changes in the activities of the pyruvate dehydrogenase complex and acetyl CoA carboxylase in rat adipose tissue. These findings indicate sites at which hormones may interact, presumably indirectly, with the pathway of fatty acid synthesis from carbohydrate. The rat fat-cell, besides carrying out de novo synthesis of fatty acids is both capable of fatty acid esterification and glyceride lipolysis. Although there is a considerable body of knowledge concerning the hormonal regulation of the latter process [9,10], information concerning interactions of hormones with the esterification process is sparse. In this investigation we have attempted to detect changes in the ability of fat-cell extracts to acylate glycerol 3-phosphate as a result of prior exposure of the cells to insulin or adrenaline.

2. Materials and methods

Male Sprague–Dawley rats weighing 160–190 g were used throughout. Chemicals were obtained and treated as described previously [11,12]. In addition L-glycerol-3-phosphate (dicyclohexylammonium salt) was obtained from Boehringer Corp. Isolated fat-cells were prepared by the method of Rodbell [13]. Cells from twelve epididymal fat pads were suspended in a final volume of 10 ml in Krebs–Ringer bicarbonate containing 10 mg of albumin per ml. 2 ml portions of this stock cell suspension were then dispensed into siliconised 50 ml flasks and incubated with shaking at 37°C in a total volume of 16 ml under an atmosphere of O_2 + CO_2 (95:5%). After 60 min the contents of incubation flasks were transferred to siliconised homogeniser tubes, centrifuged for 20 sec at approximately 200 g_{av}, the resulting infranatants removed by aspiration and the tubes containing fat-cells plunged into liquid N_2. Cells were stored under liquid N_2 for up to 4 h before extraction and enzyme assay.

Extracts from frozen fat-cells were prepared by homogenisation in 5 ml of ice-cold 0.25 M sucrose medium containing 1 mM EDTA, 1 mM dithiothreitol, 10 mM Tris-chloride buffer (pH 7.4) in an Ultra-Turrax homogeniser at 4°C for four 15 sec periods over 2 min. The extracts were centrifuged at 1000 g_{av} for 70–75 sec at 4°C, and the resulting fat-free homogenates used directly for enzymatic assays.

Glutamate dehydrogenase (EC 1.4.1.2) and lactate dehydrogenase (EC 1.1.1.27) were assayed as described previously [14].

Pyruvate dehydrogenase *a* (EC 1.2.4.1.) was assayed in a spectrophotometric assay coupled with pigeon liver arylamine acetyltransferase [2]. To correct for possible incomplete recoveries of cells after incubation, or for incomplete breakage of mitochondria during homogenisation, pyruvate dehydrogenase *a* activity was expressed as μmoles of acetyl CoA formed/min per unit of glutamate dehydrogenase.

Acyl CoA: L-glycerol 3-phosphate-*O*-acyltransferase activity (EC 2.3.1.15) was assayed at 30°C in a final volume of 1.0 ml containing 0.1 M Tris-chloride buffer pH 7.4, 0.2 μCi of [U-^{14}C] L-glycerol 3-phosphate, 65 μM palmitoyl CoA, 1.75 mg of fatty-acid poor albumin, 0.7 mM dithiothreitol and the indicated L-glycerol 3-phosphate concentrations. The reaction was initiated with 0.1 ml of tissue extract. Assays were usually performed at 4 and 8 min for each

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glycerol 3-phosphate concentration used. The assay was linear with time over this interval. The reaction was terminated with 2 ml of water-saturated butanol and radioactive incorporation into butanol-soluble products determined as described by Daae and Bremer [15]. The product of this assay gave a single radioactive spot in a thin-layer chromatographic-separation using chloroform–methanol–conc. HCl (87:13:0.5, v/v/v) which corresponded with an sn-1,2,dipalmitoyl glycerol 3-phosphate marker. Appropriate blanks were conducted in parallel with all experiments. Glycerol phosphate acyltransferase activity was expressed as nmoles of L-glycerol 3-phosphate incorporated into lipid/min per unit of lactate dehydrogenase in order to correct for any possible incomplete recovery of cells after incubation or for incomplete cell breakage during homogenisation.

Unesterified fatty acids and glycerol in incubation media were assayed as described by Itaya and Ui [16] and Garland and Randle [17] respectively.

Where appropriate, statistical differences between experimental values were *t*-tested on the basis of paired differences.

3. Results and discussion

The basic approach adopted was that if brief exposure of fat cells to hormones may modify the activities of enzymes involved in glycerol phosphate acylation, any such changes may persist through freeze-stop and extraction procedures and still be observed provided assays are performed immediately. This makes no supposition concerning the nature of any activity modifications observed.

If exposure of cells to insulin were to cause alterations in glycerol phosphate acylation activity, it was expected that these would be increases in activity [18,19]. With the experimental conditions described in fig.1 insulin indeed slightly elevated the activity with respect to insulin-free controls. This increase was significant on a paired-difference basis when assays were performed with 1.0 mM glycerol phosphate ($P < 0.025$). However, when fructose was included in similar incubations of cells (fig.2a) insulin consistently decreased glycerol phosphate acyltransferase activity ($P < 0.01$ at 0.2 mM glycerol phosphate, $P < 0.025$ at 0.3, 0.5 and 0.7 mM glycerol phosphate). When glucose was present in incubations of cells (fig.2b), no significant effects were seen with insulin. In the same experiments, incubation with fructose alone increased glycerol phosphate acyltransferase activity compared to controls incubated with no substrates (17% and 30% stimulations at 0.5 and 0.7 mM glycerol phosphate respectively, $P < 0.05$ in both cases). Insulin therefore caused small, but significant, changes in glycerol phosphate acyltransferase activity. However, the direction of these changes was influenced by the presence of fructose which itself had a positive effect upon enzyme activity. The physiological significance of these insulin effects, particularly in the presence of fructose is unclear at present.

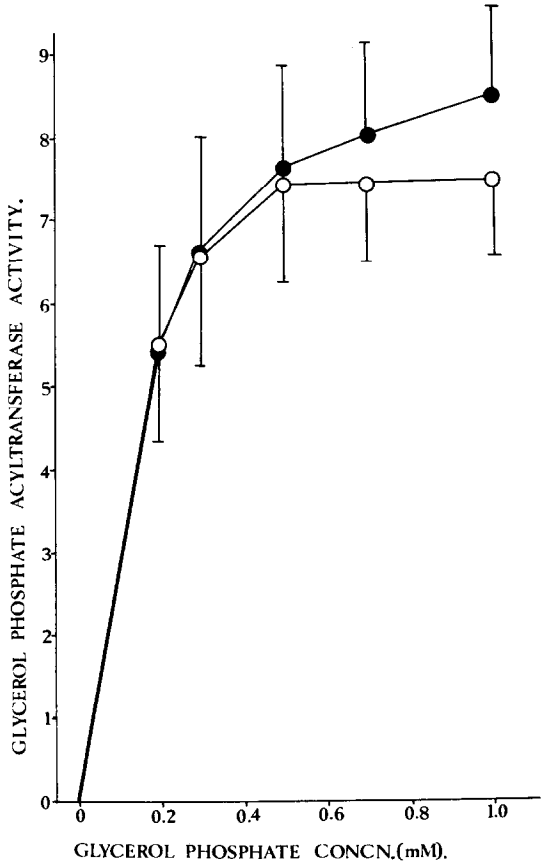

Fig.1. Fat-cells were incubated without added substrates for 1 h in Krebs–Ringer bicarbonate containing 18.8 mg of albumin/ml with (•) or without (○) insulin (20 m unit/ml). The results are means and S.E.M. of 6 experiments using 6 cell preparations. The mean fat-cell DNA was 9.8 μg/ml of incubation medium and the mean lactate dehydrogenase activity was 1.83 units/100 μg DNA.

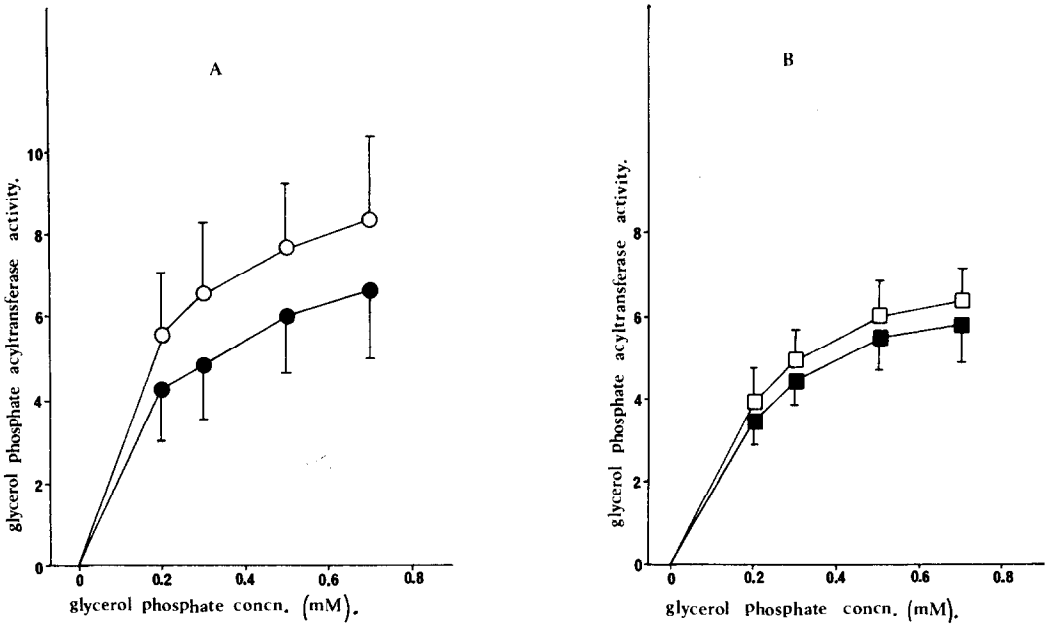

Fig.2. Fat-cells were incubated for 1 h in Krebs-Ringer bicarbonate containing 18.8 mg of albumin/ml with (closed symbols) or without (open symbols) insulin (20 munit/ml). The results are means and S.E.M. of 5 experiments using 5 cell preparations. The mean fat-cell DNA was 7.2 μg/ml of incubation medium and the mean lactate dehydrogenase activity was 1.76 units/100 μg DNA. (A) Incubation with 5 mM fructose. (B) Incubation with 5 mM glucose.

Incubation of fat-cells without a carbohydrate substrate in the presence of less than 1 μM adrenaline caused quite large decreases in glycerol phosphate acyltransferase activity (fig.3). As shown previously with fat pads [2], adrenaline also decreased pyruvate dehydrogenase *a* activity in these extracts. A concomitant stimulation of lipolysis also occurred. This is shown by the adrenaline-dependent accumulation of unesterified fatty acids in the incubation media which shows a roughly inverse profile to the measured enzyme activities. Glycerol accumulation showed a

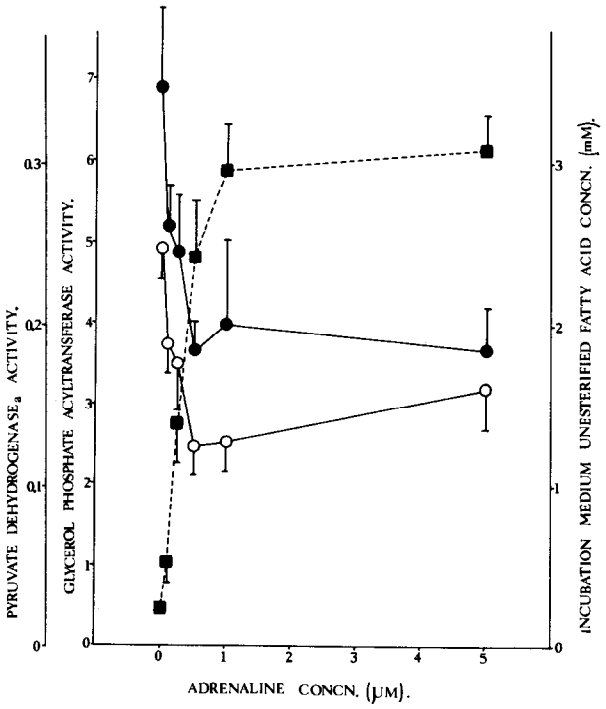

Fig.3. Fat-cells were incubated without added substrates for 1 h in Krebs–Ringer bicarbonate containing 38.8 mg of albumin/ml and adrenaline concentrations as indicated. The results are means and S.E.M. of 4 experiments. The mean fat-cell DNA was 9.8 μg/ml of incubation medium and the mean lactate dehydrogenase and glutamate dehydrogenase activities were 2.18 and 0.16 units/100 μg DNA respectively. (●) Glycerol phosphate acyltransferase activity assayed at 0.5 mM glycerol 3-phosphate; (○) pyruvate dehydrogenase *a* activity; (■) extracellular unesterified fatty acid concentration.

similar profile. From these data it is reasonable to infer that related signals may be responsible for inversely regulating triglyceride synthesis and breakdown in the adrenaline-stimulated fat cell. The possible analogies with glycogen metabolism are clear. Similarly, the unknown intracellular signals responsible for adrenergic regulation of fat-cell pyruvate dehydrogenase may be related to those regulating glycerol phosphate acylation.

Pyruvate dehydrogenase *a* activities were also routinely measured in the tissue extracts prepared for the experiments described in figs.1 and 2. Insulin increased pyruvate dehydrogenase *a* activity by an average of 21% ($P < 0.05$) in cells incubated with no substrate. Addition of fructose also increased pyruvate dehydrogenase *a* activity by 19% ($P < 0.05$). However, in the presence of fructose, insulin increased pyruvate dehydrogenase activity by an average of 18% ($P < 0.01$) which may be contrasted with the effect of the hormone in depressing glycerol phosphate acyltransferase activity under these conditions (fig.2). Hormonal effects upon pyruvate dehydrogenase and glycerol phosphate acyltransferase activities therefore do not appear to be linked under all conditions.

We conclude that relatively brief exposure of fat cells to adrenaline or insulin results in significant alternations in glycerol phosphate acyltransferase activity. The physiological appropriateness of the adrenaline effects are fairly clear, whereas the effects of insulin are less easy to interpret at present.

Acknowledgements

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